EFFECTS OF GYPSY MOTH (LEPIDOPTERA: LYMANTRIIDAE) LABORATORY STRAIN AND CROWDING ON EMERGENCE OF THE PARASITOID *COTESIA MELANOSCELUS* (HYMENOPTERA: BRACONIDAE)[1]

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ABSTRACT: We examined the success of the gypsy moth larval endoparasitoid *Cotesia melanoscelus* among 3 host rearing methods, and 2 laboratory *Lymantria dispar* host strains. When reared individually, parasitism success was higher for hosts reared in 1-oz. cups than in cells of 50-cell rearing trays. Under these conditions there was no difference in *C. melanoscelus* success between strains. However, when parasitized gypsy moths were reared together in groups of 50, there was a distinct difference in parasitoid suitability between strains. Percent larval parasitoid emergence was 7.9x higher for Delaware strain *L. dispar* larvae than for NJSS larvae. Parasitoid sex ratios were not significantly affected by host strain. The reduced ability of parasitoids to successfully develop was caused almost entirely by host mortality prior to parasitoid emergence. Implications for biological control of gypsy moth by *C. melanoscelus* are briefly discussed.

The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), is an invasive species that feeds on over 300 species of trees and shrubs (Liebhold et al. 1995). It is expanding its range south and west to new forest regions despite extensive control efforts (McFadden and McManus 1991). A number of hymenopteran and dipteran parasitoids have been introduced in North America in attempts to control the gypsy moth. Among the more important is *Cotesia melanoscelus* (Ratzburg) (Hymenoptera: Braconidae) a parasitoid of early-instar gypsy moth larvae. Development of *C. melanoscelus* from oviposition to adult emergence requires approximately 20 days, and host larvae successfully parasitized by *C. melanoscelus* die several days after parasitoid emergence (Crossman 1922). Although *C. melanoscelus* can impact low density gypsy moth populations (Barbosa et al. 1975), incomplete synchronization with the gypsy moth life cycle (Weseloh 1976), and the impact of hyperparasitoids (Muesebeck and Dohanian 1927), have been offered as explanations for its limited efficacy. In addition, *C. melanoscelus* is difficult to mass rear, as laboratory colonies often result in low levels of parasitoid emergence and highly male-biased sex ratios.

A variety of host factors can affect the quality of host insects for parasitoid development (Vinson and Iwantsch 1980; Godfray 1994). The tree species on which host larvae feed can affect *C. melanoscelus* size, development time, and

[1] Received April 26, 2001. Accepted October 18, 2001

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survival (Werren et al. 1992; Kruse and Raffa 1997). However, comparisons of *C. melanoscelus* success among different strains of its host have not been conducted. Such variation has been shown to be important for other endoparasitoids (Chabora and Chabora 1971; Carton and Nappi 1991; Henter and Via 1995; Stiling and Rossi 1996; Johnson et al. 1997). The purpose of this study was to compare different methods of laboratory host rearing and the effects of host strain on *C. melanoscelus* to improve laboratory production.

MATERIALS AND METHODS

We obtained gypsy moth egg masses from USDA-APHIS, Otis AFB, MA (strain NJSS), and USDA-ARS, Beneficial Insects Introduction Lab, Newark, DE. Insects were reared using previously described methods (Kruse and Raffa 1997). Before hatching, pre-chilled egg masses were surface sterilized in a sodium hypochlorite solution (2060 ml dH_2O, 21 ml polyoxythylene sorbitan monooleate and 40 ml bleach) for five minutes, rinsed three times with dH_2O, and allowed to dry. Larvae were reared through the 1st stadium on artificial diet (ICN Biomedicals, Aurora, OH) in circular 14.0 x 3.9 cm clear plastic rearing containers (TriState Plastics, Dixon, KY), under a 16:8 (L:D) photoperiod at 24° C and 50-70% r.h. in an environmental growth chamber. We obtained adult Korean strain *C. melanoscelus* from the USDA, Newark, DE. Wasps were maintained in culture on gypsy moth larvae and kept under a 18:6 (L:D) photoperiod at 24° C and 50-70% r.h. Newly emerged wasp cocoons were placed in 240 ml plastic cups with honey and water. After emerging as adults, wasps were allowed to mate for 24 h., and females were removed with an aspirator, placed in plastic cups, and kept in a growth chamber at 15° C until needed for experiments.

Upon molting to the 2nd stadium, randomly selected gypsy moth larvae were individually introduced into a petri dish with 2-3 mated *C. melanoscelus* females for parasitization. Under constant observation, each larva was attacked by one female and removed from the dish. A maximum of 20 larvae were parasitized by each wasp. After parasitization, larvae were randomly assigned to 1 of 3 different rearing methods: 1) individual larvae in cells (4 x 2.5 x 1.5 cm) of 50-cell clear plastic rearing trays. Trays were covered with mylar plastic, with 3 pin holes punched through for each cell (Kleiner et al. 1995; Chenot and Raffa 1998); 2) individual larvae in 1-oz. clear plastic cups with cardboard covers; 3) groups of 50 larvae in circular 14.0 x 3.9 cm clear plastic rearing dishes. Unparasitized gypsy moth larvae from the NJSS strain were also reared in groups of 50 as above for comparison. All larvae were provided with artificial diet, which was replaced every 2-3 days for larvae reared in cups and dishes, and every 7 days in trays. Larvae were monitored daily for the presence of emerged *C. melanoscelus* cocoons, gypsy moth mortality, or pupation.

Parasitism rates are expressed as percent of hosts from which a parasitoid

cocoon emerged (% larval emergence), and percent of all hosts from which an adult parasitoid was ultimately produced (% adult emergence). Percent non-emergence mortality of hosts was calculated as the original number of parasitized hosts, minus the sum of the number of parasitoid cocoons and the hosts that survived to pupation, divided by the original number of hosts. For the larvae reared individually in trays and 1-oz. cups, % parasitism, % successful parasitism, and % female parasitoids were analyzed using chi-square tests (df_1, $P = 0.05$) to determine differences between proportions among gypsy moth strains and rearing method combinations. For the larvae reared collectively in dishes, % larval emergence, % adult emergence, parasitoid non-emergence mortality, and % female parasitoids were analyzed as one-way analysis of variance (PROC GLM; SAS Institute 1990), with data from each dish as the unit of replication. Gypsy moth mortality over time for both parasitized strains and unparasitized NJSS were analyzed with repeated measures analysis of variance (PROC MIXED; SAS Institute 1990). Residuals were examined for normality using PROC UNIVARIATE (SAS Institute 1990).

RESULTS AND DISCUSSION

Percent parasitism, larval emergence, percent successful parasitism, adult emergence, and sex ratio of parasitoids emerging from gypsy moth larvae are shown in Table 1. Hosts reared in 1 oz. cups were 2.9x more likely to produce parasitoid cocoons and 5.4x more likely to produce parasitoid adults than hosts reared in trays. When hosts were reared individually, there was no difference in parasitoid success between the two host strains. Sex ratios were not significantly affected by rearing method, or host strain.

When hosts were reared in groups of 50, there was a strong effect of host strain on the success of *C. melanoscelus* (Table 2). Percent parasitism was 7.9x higher, and percent successful parasitism 11.5x higher for Delaware strain larvae than for NJSS larvae. Sex ratios were not significantly affected by host strain. The non-emergence mortality of host larvae was 56.41% higher for NJSS strain larvae than Delaware strain. By day 14, when parasitoids began emerging, there were was an average of 39.2 hosts alive per dish out of the original 50 for the Delaware NJSS strain and 13.6 alive for the Delaware NJSS strain (Fig. 1).

The success of *C. melanoscelus* parasitism was affected by both rearing method and host strain. When reared individually, hosts in 1-oz. cups produced significantly more parasitoids than hosts reared in 50-cell trays. This may be related to more frequent replenishment of fresh diet, or less humid conditions in cups than trays.

When reared individually, there was no difference in parasitoid success between the two gypsy moth strains. However, a distinct difference in host suitability was evident between strains when larvae were reared in groups of 50 (Table 2, Fig. 1). Crowding in field and laboratory populations can cause

Table 1. Effects of gypsy moth strain and rearing method on success of parasitism by *C. melanoscelus* for individually reared hosts. Means followed by different letters within a column are significantly different (Chi-square test df_1, $P < 0.05$).

Gypsy Moth Strain	Rearing Method	N	% Larval Emergence	% Adult Emergence	N	% Female Parasitoids
Delaware	Trays	79	15.19 a	6.33 a	5	20.00 a
NJSS	Trays	78	16.67 a	5.13 a	4	25.00 a
Delaware	1 oz. cups	60	51.67 b	26.67 b	16	12.50 a
NJSS	1 oz. cups	60	41.67 b	35.00 b	21	9.52 a

Table 2. Effect of gypsy moth strain on the success of parasitism by *C. melanoscelus* for hosts reared in groups of 50. Means ± SE followed by different letters are significantly different (Fisher's protected LSD, $P < 0.05$).

Gypsy Moth Strain	N	% Larval Emergence	% Adult Emergence	% Non-Emergence Mortality	N	% Female Parasitoids
Delaware	13	65.85+7.41 a	27.23+2.35 a	29.23+6.11 a	177	32.20+3.89 a
NJSS	11	8.36+2.19 b	2.36+0.68 b	85.64+2.17 b	13	42.43+13.34 a

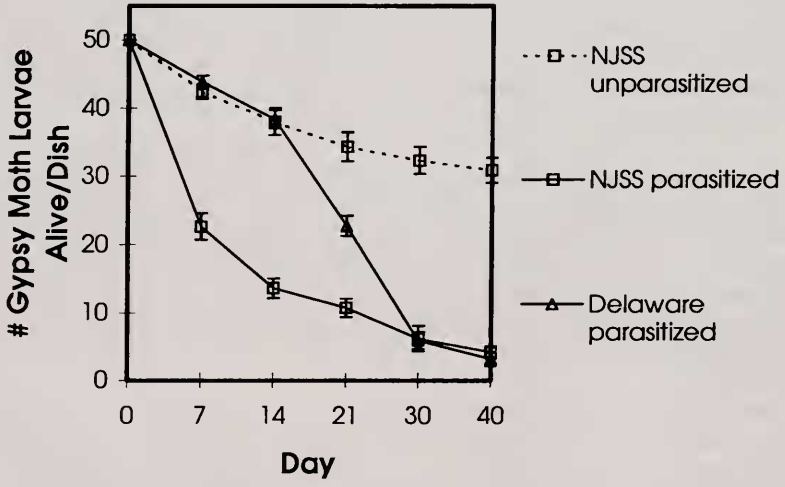

Figure 1. Mortality for two laboratory strains of parasitized gypsy moth larvae reared in groups of 50. Experiment began when larvae were parasitized upon molting to the second instar (day 0). Error bars indicate standard errors for each day.

physiological changes in gypsy moth larvae (Leonard 1981). The effects of crowding on larval development may differ between the two strains and thus differentially affect the suitability of larvae for *C. melanoscelus*. The effects of crowding on parasitoid success will require further experimentation in both the laboratory and the field.

Previous dissections of randomly selected laboratory-reared gypsy moth larvae that had been exposed to *C. melanoscelus* demonstrated that parasitoid larvae were almost always alive prior to the death of their host (Chenot 1996). This suggests that the failure of parasitoids to successfully develop is more likely due to general features of host quality than active host defenses. The results reported here are consistent with this conclusion. The reduced ability of parasitoids to successfully develop in NJSS strain hosts was caused almost entirely by non-emergence from hosts that died prematurely (Table 2, Fig. 1). Survival of unparasitized NJSS larvae was the same as parasitized Delaware larvae until day 14, when parasitoid larvae started to emerge, while parasitized NJSS larvae died at significantly higher rate. By day 14, 78.4% of parasitized Delaware strain gypsy moths were still alive compared to only 27.2% of parasitized NJSS larvae.

These bioassays revealed significant genotypic variation in parasitoid suitability among gypsy moth strains. The NJSS laboratory strain has been in colony for over 35 generations. Over time, phenotypic changes such as faster development times, higher fecundity, increased resistance to nuclear polyhedrosis virus (NPV), and decreased resistance to the bacteria *Bacillus thuringiensis* (Bt) have been observed (Keena and O'Dell 1994). Laboratory colonies often experience significant inbreeding and are under different selective pressures than wild populations. However, variability between laboratory colonies suggests similar variability could exist in field populations. This effect could impact biological control efforts and could help explain the historically limited success of *C. melanoscelus* in reducing gypsy moth populations.

ACKNOWLEDGMENTS

We thank A. Chenot for her work that initiated this project. M. Clayton (UW-Madison Dept. Statistics) provided statistical guidance, G. Bernon (USDA-APHIS) provided gypsy moth egg masses, and R. Fuester and K. Swan (USDA-ARS-BIIL) provided gypsy moth egg masses and *C. melanoscelus*. We thank M. Keena (USDA Forest Service), and D. Hogg and M. Strand (UW-Madison Department of Entomology) for critical review of this manuscript. This work was supported by the Wisconsin Department of Natural Resources, McIntire-Stennis, and the University of Wisconsin-Madison College of Agricultural and Life Sciences.

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